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(54) Title: AN ENZYME FOR DYEING KERATINOUS FIBRES

(57) Abstract

The present invention relates to a dyeing composition comprising an oxidation enzyme derived from the genus *Pyricularia*, a method for dying hair, and the use of a *Pyricularia* laccase for dyeing keratinous fibres.

Title: An enzyme for dyeing keratinous fibres

FIELD OF THE INVENTION

The present invention relates to a dyeing composition for keratinous fibres, such as hair, a method for dyeing keratinous fibres and the use of an oxidation enzyme derived from *Pyricularia* for dyeing keratinous fibres, such as human or animal hair.

BACKGROUND OF THE INVENTION

It has been used for many years to dye the hair of humans to cover appearing grey hair. The need to do so arises from the fact that grey hair is the first sign of having past adolescence, which can be hard to accept for many people.

Further, during the last few decades hair dyeing has become more and more popular in the western world. At first Punk Rockers and other society critical groups dyed their hair in extreme colours as a part of their protest against the established society, but today especially many young people also use hair dyes (in more soft tints than the Punk Rockers) as a sort of "cosmetic" to change or freshen up their "looks".

Hair dyes

In general hair dyeing compositions on the market today can be divided into three main groups:

- temporary hair dyes,
- semi-permanent hair dyes, and
- permanent oxidative hair dyes.

The temporary hair dyes are only intended to change the natural hair colour for a short period of time and usually function by depositing dyes on the surface of the hair. Such hair dyes are easy to remove with normal shampooing.

When using semi-permanent hair dyes the colour of the dyed hair can survive for five or more shampooings. This is achieved by using dyes which have a high affinity for hair keratin and which are capable of penetrating into th interior

also dye precursors, such as bases and couplers, in a buffer solution. The pH in said composition li s between 6.5 and 8 and said enzyme has an optimal activity in the pH range between 6.5 and 8.

Rhizoctonia praticola laccase and *Rhus vernicifera* laccase have a pH-optimum between 6.5 and 8 and can be used to form the polymeric dyes according to this patent.

WO 95/33836 (Novo Nordisk A/S) describes the use of a laccase derived from *Myceliopthora thermophila* which may be used for dyeing of hair.

WO 96/00290 (Novo Nordisk A/S) discloses the use of a laccase derived from *Polyporus pinsitus* for oxidative dyeing of hair.

It is known that *Pyricularia oryzae* laccase may be used oxidation of phenolic azo dyes (see Muralikrishna et al., (1995), Appl. Environ. Microbiol., 61, (12), pp. 4374-4377).

The use of *Pyricularia* laccase for dyeing keratinous materials such as hair is not mentioned and anticipated by said document.

SUMMARY OF THE INVENTION

The object of the present invention is to provide a permanent dyeing composition for keratinous fibres, such as hair, which has an improved colour development (*i.e.* dyeing effect).

The terms "colour development" and "dyeing effect" are used interchangeably in the following defining a colour change (measured as DE) of the dyed keratinous fibre in question.

It has surprisingly been found that it is possible to provide such an improved hair dyeing composition by using a laccase derived from a strain of the filamentous fungus genus *Pyricularia* as the oxidation enzyme.

Firstly, the invention relates to a permanent dyeing composition for keratinous fibres, in particular hair, comprising an oxidation enzyme comprising

1) one or more oxidation enzymes derived from a strain of the genus *Pyricularia*,

effect for *Pyricularia* laccase is higher than for *Polyporus* laccase.

Improved colour development is, in the context of the present invention, defined as a DE higher than the DE value of the above mentioned *Polyporus pinsitus* laccase.

Consequently, in the first aspect the present invention relates to a permanent dye composition for keratinous fibres, such as hair, comprising

1) one or more oxidation enzymes derived from a strain of the genus *Pyricularia*,

2) one or more dye precursors, and

optionally 3) one or more modifiers.

In an embodiment of the invention the oxidation enzyme is a laccase derived from a strain of genus *Pyricularia*, such as a strain of *Pyricularia oryzae* e.g. the laccase which can be purchased from SIGMA under the trade name SIGMA no. L5510.

In addition, laccases derived from the genus *Pyricularia* also encompass alternative forms of laccases which may be found in *Pyricularia* as well as laccases which may be found in other fungi which are synonyms or fall within the definition of the genus *Pyricularia*.

It is to be understood that the *Pyricularia* laccases used for dyeing keratinous fibres according to the present invention may be produced homologously or heterologously using especially filamentous fungi, yeasts or bacteria as host cells.

Examples of filamentous fungus host cells include strains of the species of *Trichoderma*, preferably a strain of *Trichoderma harzianum* or *Trichoderma reesei*, or a species of *Aspergillus*, most preferably *Aspergillus oryzae* or *Aspergillus niger*, or yeast cells, such as e.g. a strain of *Saccharomyces*, in particular *Saccharomyces cerevisiae*, *Saccharomyces kluyveri* or *Saccharomyces uvarum*, a strain of *Schizosaccharomyces* sp., such as *Schizosaccharomyces pombe*, a strain of *Hansenula* sp.,

The dye precursor is preferably an aromatic compound e.g. belonging to one of three major chemical families: the diamines, aminophenols (or aminonaphtols) and the phenols.

Examples of such suitable dye precursors include compounds from the group comprising comprising p-phenylene-diamine (pPD), p-toluylene-diamine (pTD), chloro-p-phenylenediamine, p-aminophenol, o-aminophenol, 3,4-diaminotoluene, 2-methyl-1,4-diaminobenzene, 4-methyl-o-phenylenediamine, 2-methoxy-p-phenylenediamine, 2-chloro-1,4-diamino-benzene, 4-amino diphenylamine, 1-amino-4-β-methoxyethylamino-benzene, 1-amino-4-bis-(β-hydroxyethyl)-amonibenzene, 1-3-diamino-benzene, 2-methyl-1,3-diamino-benzene, 2,4-diaminotoluene, 2,6-diaminopyridine, 1-hydroxy-2-amino-benzene, 1-hydroxy-3-amino-benzene, 1-methyl-2-hydroxy-4-amino-benzene, 1-methyl-2-hydroxy-4-β-hydroxyethylamino-benzene, 1-hydroxy-4-amino-benzene, 1-hydroxy-4-methylamino-benzene, 1-methoxy-2,4-diamino-benzene, 1-ethoxy-2,3-diamino-benzene, 1-β-hydroxyethyloxy-2,4-diamino-benzene, phenazines, such as 4,7-phenazinedicarboxylic acid, 2,7-phenazinedicarboxylic acid, 2-phenazinecarboxylic acid, 2,7-diaminophenazine, 2,8-diaminophenazine, 2,7-diamino-3,8-dimethoxyphenazine, 2,7-diamino-3-methoxyphenazine, 2,7-diamino 3-methoxyphenazine, 3-dimethyl 2,8-phenazinediamine, 2,2'-[(8-amino-7-methyl-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-methoxy-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-chloro-2-phenazinyl)imino]bis-ethanol, 2-[(8-amino-7-methyl-2-phenazinyl)amino]-ethanol, 2,2'-[(8-amino-2-phenazinyl)imino]-bis-ethanol, 3-amino-7-(dimethylamino)-2,8-dimethyl-5-phenyl-chloride, 9-(diethylamino)- benzo[a]phenazine-1,5-diol, N-[8-(diethylamino)-2-phenazinyl]- methanesulfonamide, N-(8-methoxy-2-phenazinyl)-methanesulfonamide, N,N,N',N'-tetramethyl-2,7-phenazinediamine, 3,7-dimethyl-2-phenazinamine, p-amino benzoic acids, such as p-amino benzoic acid ethyl, p-amino benzoic acid glycerid, p-amino benzoic acid isobutyl, p-dimethylamino benzoic acid amil, p-dim thylamino benzoic acid octyl, p-diethoxy amino benzoic amil, p-dipropoxy amino

conditions sufficient to permit oxidation of the dye precursor.

The dyeing method can be conducted with one or more dye precursors, either alone or in combination with one or more modifiers. Amounts of components are in accordance with usual commercial amounts for similar components, and proportions of components may be varied accordingly.

When using an oxidation enzyme derived from *Pyricularia*, such as the *Pyricularia oryzae* laccase mentioned above, the method for dyeing hair of the invention may be carried out at room temperature and at a pH in the range from 5.0 to 9.0, preferably 6.0 to 8.0, especially around pH 7.

Suitable dye precursors and optionally modifiers are described above.

The use of an oxidative enzyme derived from *Pyricularia*, such as a laccase, is an improvement over the more traditional use of H_2O_2, in that the latter can damage the hair, and its use usually requires a high pH, which is also damaging to the hair. In contrast, the reaction with an enzyme can be conducted at acidic or neutral pH (below pH 9.0), and the oxygen needed for oxidation comes from the air, rather than via harsh chemical oxidation.

The result provided by the use of the oxidation enzyme derived from *Pyricularia*, such as a laccase, is comparable to that achieved with use of H_2O_2, not only in colour development, but also in wash stability and light fastness. An additional commercial advantage is that a single container package can be made containing both the laccase and the precursor, in an oxygen free atmosphere, which arrangement is not possible with the use of H_2O_2.

Also when comparing the colour development using an oxidation enzyme derived from the genus *Pyricularia*, such as a laccase, with a laccase such as the *Polyporus* laccase described above the *Pyricularia* oxidation enzym gives improved colour development.

Assessment of the hair colour

The quantitative colour of the hair tresses are determined on a Minolta CR200 Chroma Meter by the use the parameters L* ("0"=black and "100"=white), a* ("-60"=green and "+60"=red) and b* ("-60" blue and "+60" yellow).

DL*, Da* and Db* are the delta values of L*, a* and b* respectively compared to L*, a* and b* of untreated hair (*e.g.* $DL^* = L^*_{sample} - L^*_{untreated\ hair}$).

DE* is calculated as $DE^*=\sqrt{(DL^{*2}+Da^{*2}+Db^*)}$ and is an expression for the total quantitative colour change (*i.e.* colour development or dyeing effect).

EXAMPLES

Example 1

Dyeing effect

The dyeing effect of a *Pyricularia oryzae* laccase was tested using the dye precursor p-phenylenediamine and compar d with an equivalent activity of *Polyporus pinsitus* laccase under the same reaction conditions.

Hair dyeing

1 gram De Meo white hair tresses were used.

4 ml dye precursor solution was mixed with 1 ml laccase on a Whirley mixer, applied to the hair tresses and incubated at 30°C for 60 minutes. The activity of both the *Pyricularia oryzae* laccase and the *Polyporus pinsitus* laccase were 0.048 LACU/ml reaction mixture (pH 7).

The hair tresses were then rinsed with running water, washed with shampoo, rinsed with running water, combed, and air dried.

The a*, b* and L* was determined on the Chroma Meter and the DE* values were then calculated.

A hair tress sample treated without enzyme was used as a blind.

a*, b* and L* were measured on the Chroma Meter and ΔE* was then calculated.

Hair tress samples treated without enzyme were used as blinds.

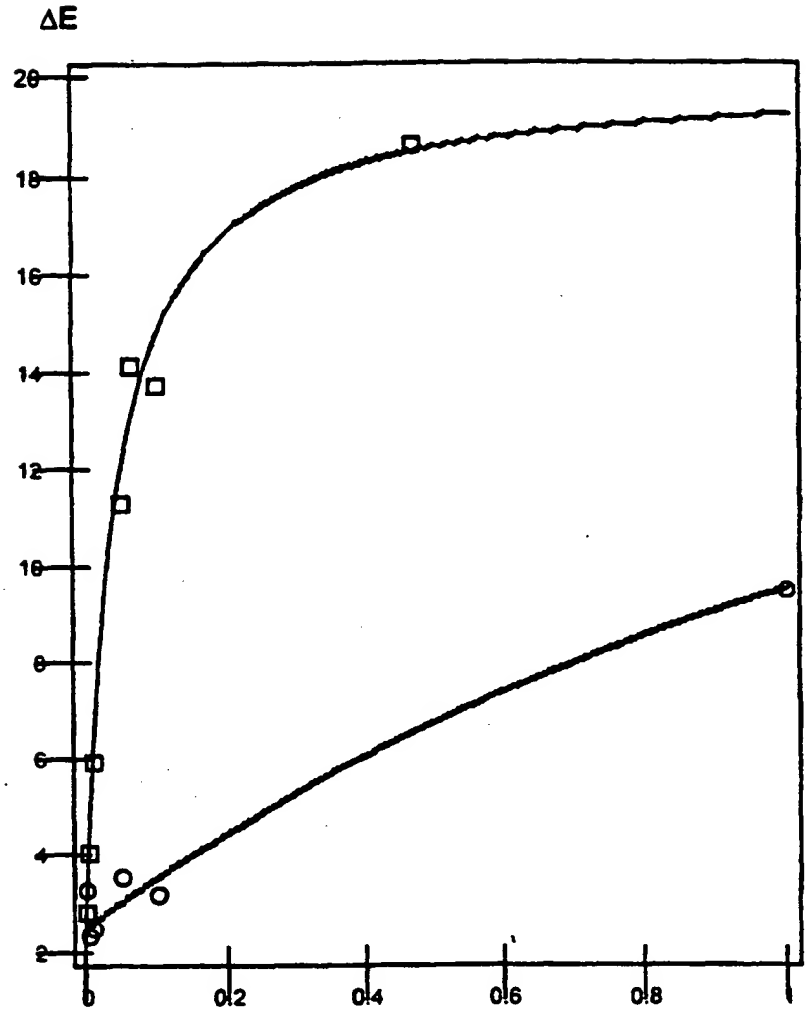
The result of the test is displayed in Figure 1.

From Figure 1 it can be seen that the *Pyricularia* laccase gives a higher ΔE value than the *Polyporus* laccase at equivalent LACU/ml reaction mixture.

phenazinedicarboxylic acid, 2-phenazinecarboxylic acid, 2,7-diaminophenazine, 2,8-diaminophenazine, 2,7-diamino-3,8-dimethoxyphenazine, 2,7-diamino-3-methoxyphenazine, 2,7-diamino 3-methoxyphenazine, 3-dimethyl 2,8-phenazinediamine, 2,2'-[(8-amino-7-methyl-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-methoxy-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-chloro-2-phenazinyl)imino]bis-ethanol, 2-[(8-amino-7-methyl-2-phenazinyl)amino]-ethanol, 2,2'-[(8-amino-2-phenazinyl)imino]bis-ethanol, 3-amino-7-(dimethylamino)-2,8-dimethyl-5-phenyl-chloride, 9-(diethylamino)- benzo[a]phenazine-1,5-diol, N-[8-(diethylamino)-2-phenazinyl]- methanesulfonamide, N-(8-methoxy-2-phenazinyl)-methanesulfonamide, N,N,N',N'-tetramethyl-2,7-phenazinediamine, 3,7-dimethyl-2-phenazinamine, p-amino benzoic acids, such as p-amino benzoic acid ethyl, p-amino benzoic acid glycerid, p-amino benzoic acid isobutyl, p-dimethylamino benzoic acid amil, p-dimethylamino benzoic acid octyl, p-diethoxy amino benzoic amil, p-dipropoxy amino benzoic acid ethyl, acetylsalicylic acid, isatin derivatives, such as 2,3-diamino benzoic acid.

5. The permanent hair dyeing composition according to claims 3 and 4, comprising a dye modifier selected from the group comprising m-phenylene-diamine, 2,4-diaminoanisole, 1-hydroxynaphthalene(α-naphthol), 1,4-dihydroxybenzene(hydroquinone), 1,5-dihydroxynapthalene, 1,2-dihydroxybenzene(pyrocatechol), 1,3-dihydroxybenzene (resorcinol), 1,3-dihydroxy-2-methylbenzene, 1,3-dihydroxy-4-chlorobenzene (4-chlororesorcinol), 1,2,3,trihydroxybenzene, 1,2,4-trihydroxybenzene, 1,2,4-trihydroxy-5-methylbenzene, 1,2,4-trihydroxytoluene.

6. A method for dyeing keratinous fibres, such as hair, comprising contacting an oxidation enzyme, such as a laccas , derived from a strain of the genus *Pyricularia*, in the presence or absence of at least one modifier, with at least one dye precursor, for a period of time, and under conditions



Polyporus pinsitus laccase: O

Pyricularia oryzae laccase: □

Figure 1

INTERNATIONAL SEARCH

International application No.
PCT/DK 97/00145

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9600290 A1 (NOVO NORDISK BIOTECH, INC.), 4 January 1996 (04.01.96), page 48, line 25 - page 54, line 24, claims 37-48	1-12

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